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The Serum Metabolome Identifies Biomarkers of Dietary Acid Load in 2 Studies of Adults with Chronic Kidney Disease

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ABSTRACT

Background: Dietary acid load is a clinically important aspect of the diet that reflects the balance between acid-producing foods, for example, meat and cheese, and base-producing foods, for example, fruits and vegetables.

Methods: We used metabolomics to identify blood biomarkers of dietary acid load in 2 independent studies of chronic kidney disease patients: the African American Study of Kidney Disease and Hypertension (AASK, $n = 689$) and the Modification of Diet in Renal Disease (MDRD, $n = 356$) study. Multivariable linear regression was used to assess the cross-sectional association between serum metabolites whose identity was known (outcome) and dietary acid load (exposure), estimated with net endogenous acid production (NEAP) based on 24-h urine urea nitrogen and potassium, and adjusted for age, sex, race, randomization group, measured glomerular filtration rate, log-transformed urine protein-to-creatinine ratio, history of cardiovascular disease, BMI, and smoking status.

Results: Out of the 757 known, nondrug metabolites identified in AASK, 26 were significantly associated with NEAP at the Bonferroni threshold for significance ($P < 6.6 \times 10^{-5}$). Twenty-three of the 26 metabolites were also identified in the MDRD study, and 13 of the 23 (57%) were significantly associated with NEAP ($P < 2.2 \times 10^{-3}$), including 5 amino acids (S-methylmethionine, indolepropionylglycine, indolepropionate, N-methylproline, N-δ-acetylornithine), 2 cofactors and vitamins (threonate, oxalate), 1 lipid (chiro-inositol), and 5 xenobiotics (methyl glucopyranoside, stachydrine, catechol sulfate, hippurate, and tartronate). Higher levels of all 13 replicated metabolites were associated with lower NEAP in both AASK and the MDRD study.

Conclusion: Metabolomic profiling of serum specimens from kidney disease patients in 2 study populations identified 13 replicated metabolites associated with dietary acid load. Additional studies are needed to validate these compounds in healthy populations. These 13 compounds may potentially be used as objective markers of dietary acid load in future nutrition research studies. *J Nutr* 2019;149:578–585.

Keywords: metabolites, kidney disease, dietary acid load, biomarkers, dietary assessment

Introduction

Guidelines for management of chronic kidney disease recommend restricting dietary intake of individual nutrients including protein to slow kidney disease progression (1). Recent research suggests that the source of dietary protein may influence kidney disease risk (2). Specifically, consumption of red and processed meat was prospectively associated with increased risk of kidney disease, whereas consumption of nuts, legumes, and low-fat dairy was associated with reduced risk of incident chronic kidney disease (2). In addition, higher adherence to the Dietary Approaches to Stop Hypertension (DASH) diet, which consists of a high intake of fruits, vegetables, nuts, legumes, and low-fat dairy products, and a low intake of red and processed meat and sodium, was shown to be associated with a lower risk of kidney disease (3). Replacing animal sources of protein with plant sources of protein along with increased intake of fruits and vegetables is 1 proposed strategy for improving health and maintaining nutritional status on a low-protein diet (4).

Dietary acid load, or the balance of acid-producing foods (such as meats and cheese) and base-producing foods (such as fruits and vegetables), is inextricably linked to type of dietary protein and may be 1 mechanism underlying the association between dietary intake and risk for kidney disease progression (5–7). In the Atherosclerosis Risk in Communities study, we found that higher quartiles of dietary acid load, estimated by net endogenous acid production (NEAP) and potential renal acid load, were significantly associated with an elevated risk of incident chronic kidney disease (8). Among individuals with

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kidney disease and hypertension in the African American Study of Kidney Disease and Hypertension (AASK), higher quartiles of NEAP were associated with faster decline in glomerular filtration rate (GFR) (6). It has also been demonstrated in clinical trials that reducing acid load through diet modification attenuates kidney injury and slows GFR decline (9–11).

There is a critical need for new biomarkers of dietary intake as an objective means of assessing food consumption (12, 13). Dietary acid load has historically been estimated using either self-report of dietary intake, which is prone to biases, or measurement of compounds in urine specimens, which is burdensome for study participants and patients to collect (5). The blood metabolome provides readily accessible, unbiased characterization of food intake and metabolism. Relating standardly assessed dietary acid load to blood metabolites may identify new biomarkers and metabolic pathways affected by dietary acid load (14).

The objective of the present study was to identify blood biomarkers of dietary acid load in AASK with external replication in the Modification of Diet in Renal Disease (MDRD) study. We aimed to discover new and reproducible biomarkers of dietary acid load, as a clinically important and potentially modifiable risk factor.

Methods

Study design

The present study is a discovery analysis of metabolites associated with dietary acid load in AASK with replication in the MDRD study. In both studies, we performed a cross-sectional analysis. AASK was a multicenter, randomized 3 × 2 factorial trial designed to test the effect of antihypertensive medications and blood pressure control on the rate of change in GFR (15). Participants were enrolled and randomly assigned between 1995 and 1998 and were followed through 2001. The MDRD study was a randomized, 2 × 2 factorial trial on the effect of dietary protein and phosphorus intake and blood pressure on kidney disease progression, defined primarily as change in GFR (16). Participants were invited to participate in the MDRD study between 1989 and 1991, and they were followed through 1994. Approval was provided by the institutional review board and procedures were followed in accordance with the Declaration of Helsinki.

Study population

Study participants who attended the baseline visit in AASK and those who attended the 12-mo follow-up visit in the MDRD study were included in the present study. AASK participants included 1094 African-American adults aged 18–70 y from 13 states in the United States with kidney disease attributed to hypertension and GFR between 20 and 65 mL/(min · 1.73 m^2). Of the 1094 randomly assigned participants in AASK, we excluded those with missing information for urine excretion of urea nitrogen ($n = 1$), possible overcollection of urine over 24 h defined as >30% of expected creatinine excretion (22.1 mg/kg for men and 17.2 mg/kg for women) ($n = 242$), possible undercollection of urine defined as <30% of expected creatinine excretion ($n = 72$), measured glomerular filtration rate (mGFR) <20 mL/(min · 1.73 m^2), and those for whom metabolomics data were not available ($n = 87$), yielding an analytic sample size of 689 (**Figure 1**A). The definitions of possible overcollection and undercollection were the same as used in prior research (6, 17).

In the MDRD study, participants included 840 men and women aged 18–70 y with chronic kidney disease [GFR from 13 to 55 mL/(min · 1.73 m^2)] from 15 clinical centers. Among the 840 randomized participants, we excluded those who did not attend the 12-mo follow-up visit, which was when blood specimens were collected for metabolomic profiling ($n = 94$), those who possibly undercollected urine ($n = 168$), those who possibly overcollected urine ($n = 59$), those with mGFR <20 mL/(min · 1.73 m^2) ($n = 134$), those with missing mGFR ($n = 12$), and those for whom metabolomics data were not available ($n = 17$), yielding an analytic sample size for replication of 356 (Figure 1B). The rationale for excluding those with low mGFR was to increase homogeneity between the discovery and replication samples.

Estimation of dietary acid load

In both AASK and the MDRD study, 24-h urine specimens were collected from study participants. Protein intake (g/d) was calculated as: (6.25 × [urine urea nitrogen (g/d) + body weight (kg) × 0.031]) (6, 18). If urine protein excretion was ≥5 g/d, we adjusted the estimate of protein intake by subtracting urine protein from the estimate. We calculated NEAP as a measure of dietary acid load using 24-h urine excretion of urea nitrogen to estimate dietary intake of protein and 24-h urine excretion of potassium: NEAP (mEq/d) = 54.5 × [protein (g/d)/potassium (mEq/d)] − 10.2 (19).

Metabolomic profiling

Serum specimens were stored at −70°C before laboratory measurement. In both studies, metabolomic profiling was conducted by Metabolon through use of 2 reverse phase ultraperformance liquid chromatography tandem mass spectrometry with positive ion mode electrospray ionization, another reverse phase ultraperformance liquid chromatography tandem mass spectrometry method with negative ion mode electrospray ionization, and a hydrophilic interaction ultraperformance liquid chromatography tandem mass spectrometry method with negative ion mode electrospray ionization (20). Metabolomic profiling of the AASK specimens was conducted in 2017 and that of the MDRD study specimens was conducted in 2015. Metabolites were identified by matching features to a library of reference standards on the basis of retention time, mass-to-charge ratio, and chromatographic data. Levels of metabolites were quantified using the area under the curve of the mass spectrometry peaks after interday normalization. In AASK, the analysis was restricted to known, nondrug metabolites, and, in the MDRD study, the analysis was restricted to known, nondrug metabolites that were statistically significant in AASK and identified in the MDRD study.

In AASK, a total of 1228 metabolites were identified in the serum specimens, including 833 known and 395 unknown metabolites. We excluded specimens with >50% missing for all metabolites. Metabolites with >80% missing across specimens were excluded. For the remaining known, nondrug metabolites, undetectable values were imputed to the minimum measured value for each metabolite. Metabolites were then scaled to a median value of 1 and log-transformed. In addition, metabolites with a variance <0.01 and outliers (defined as values >5 SD above or below the mean) were excluded. After the preprocessing step, a total of 1194 metabolites remained, including 819 known and 375 unknown metabolites. There were 62 metabolites classified in the

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Author disclosures: Assay costs were discounted as part of a collaborative agreement between Metabolon and JC, LAI, and ASL to develop metabolomic estimates of glomerular filtration rate (GFR) and for which they have a provisional patent filed on August 15, 2014 entitled "Precise estimation of GFR from multiple biomarkers" (PCT/US2015/044567). The technology is not licensed in whole or in part to any company.

Supplemental Table 1 is available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn/.

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Abbreviations used: AASK, African American Study of Kidney Disease and Hypertension; DASH, Dietary Approaches to Stop Hypertension; GFR, glomerular filtration rate; MDRD, Modification of Diet in Renal Disease study; mGFR, measured glomerular filtration rate; NEAP, net endogenous acid production.

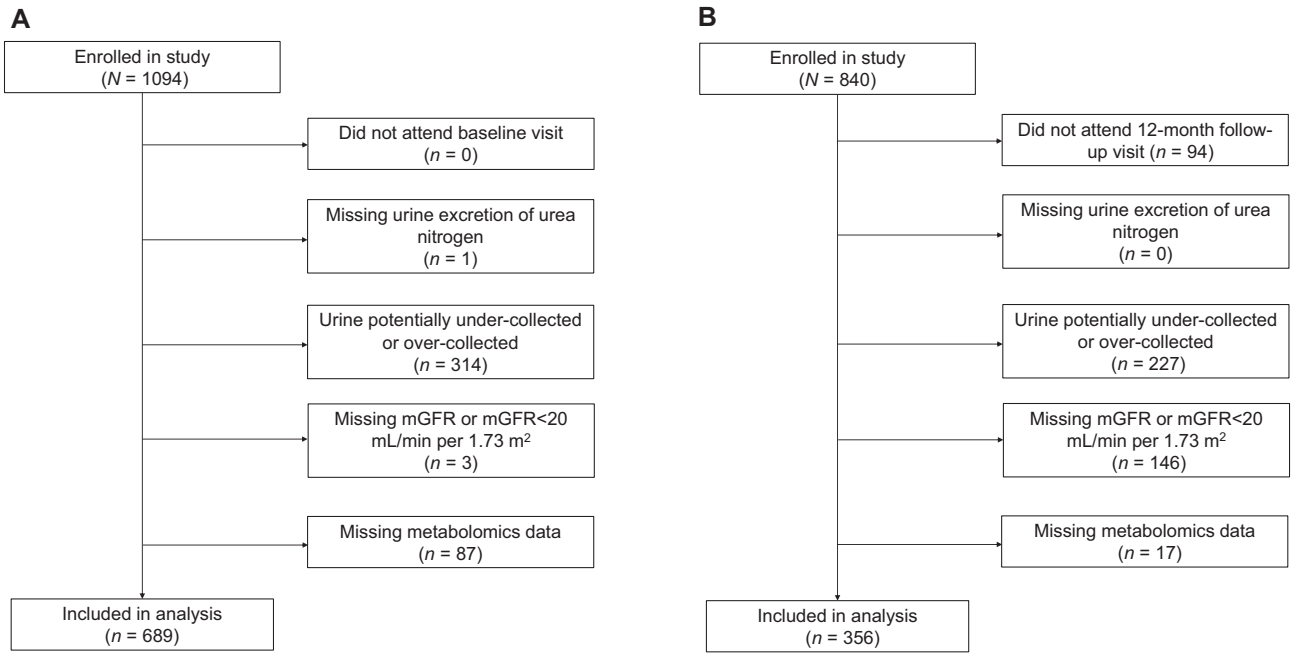

FIGURE 1 Flow chart of study participant selection in AASK (A) and the MDRD study (B). AASK, African American Study of Kidney Disease and Hypertension; MDRD, Modification of Diet in Renal Disease; mGFR, measured glomerular filtration rate.

drug subpathway that were excluded. The present analysis was limited to the remaining 757 known, nondrug metabolites.

In AASK and the MDRD study, the median correlations between metabolite concentrations for 20 blind duplicates were 0.92 and 0.91, respectively, and 86% and 72%, respectively, of metabolites had a correlation coefficient >0.80.

Covariates

GFR was measured by urinary clearance of ^{125}I-iothalamate and estimated with serum creatinine using the 2009 Chronic Kidney Disease Epidemiology equation (21, 22). Urine concentrations of urea nitrogen, potassium, protein, creatinine in 24-h urine specimens, and serum concentrations of bicarbonate and creatinine from fasting blood specimens were measured using standard procedures, as previously described (15, 16, 23).

Statistical analysis

We reported descriptive statistics (mean, standard deviation, frequencies) for baseline characteristics of study participants in AASK and the MDRD study according to tertiles of NEAP. The distribution of dietary acid load values, as quantified by NEAP, was displayed using a kernel density plot.

We investigated the cross-sectional association between each serum metabolite (outcome) and dietary acid load (exposure), estimated with NEAP, in AASK using multivariable linear regression. The main model was adjusted for demographic characteristics (age, sex, race), study design features (randomization group, which in AASK included antihypertensive medication and blood pressure target, and, in the MDRD study, included protein intake and blood pressure target), kidney measures (mGFR, log-transformed urine protein-to-creatinine ratio), and indicators of health status and health behaviors (history of cardiovascular disease, BMI, and smoking status). In addition, we ran models without adjusting for mGFR to examine the influence of this covariate on the results. The Bonferroni method was used to account for multiple comparisons, that is, in AASK, we used a threshold of 6.6×10^{-5} (0.05/757 metabolites) to assess statistical significance, and, in the MDRD study, we used a threshold of 2.2×10^{-3} (0.05/23 metabolites) (24). Estimates for metabolites that were significantly associated with NEAP in AASK and were identified in the MDRD study were pooled across the 2 studies using DerSimonian and Laird random-effects models (25). For the serum metabolites that were significantly associated with NEAP in both AASK and the MDRD study, we calculated Pearson correlation coefficients between metabolites using data from AASK. We calculated C-statistics as a measure of the cumulative ability of the significant metabolites in addition to the covariates (age, sex, race, randomization group, mGFR, log-transformed urine protein-to-creatinine ratio, history of cardiovascular disease, BMI, and smoking status) to classify participants in the highest quartile compared with the lower 3 quartiles of dietary acid load in both AASK and the MDRD study.

All analyses were conducted using Stata statistical software version 14.2 (StataCorp).

Results

In the discovery sample of 689 AASK participants, the mean age was 54 y, 37% were female, 100% were black, and mean mGFR was 46 mL/(min · 1.73 m^2). About half of AASK participants (51%) had a history of cardiovascular disease, the mean BMI was 30 kg/m^2, and 28% were current smokers. In the replication sample of 356 MDRD study participants, mean age was 52 y, 38% were female, 8% were black, and mean mGFR was 36 mL/(min · 1.73 m^2). Relative to AASK participants, a smaller proportion of MDRD study participants had a history of cardiovascular disease (6%) and were current smokers (10%), and mean BMI was lower (27 kg/m^2). In both AASK and the MDRD study, weight, urea nitrogen, and protein intake were higher and urine potassium and serum bicarbonate were lower with higher tertiles of NEAP (**Table 1**). There was no appreciable difference in mGFR and estimated glomerular filtration rate across tertiles of NEAP.

The mean (SD) NEAP was 78.4 (33.4) mEq/d in AASK and 54.0 (21.2) mEq/d in the MDRD study. The kernel density plot for NEAP demonstrated a wider range and higher values in AASK relative to the MDRD study (**Figure 2**).

Out of the 757 metabolites analyzed in AASK, a total of 26 known serum metabolites were significantly associated with

TABLE 1 Baseline characteristics of study participants in AASK and the MDRD study according to tertile of net endogenous acid production[1]

	AASK ($N = 689$)			MDRD ($N = 356$)		
Characteristic	Tertile 1 ($n = 230$)	Tertile 2 ($n = 230$)	Tertile 3 ($n = 229$)	Tertile 1 ($n = 119$)	Tertile 2 ($n = 119$)	Tertile 3 ($n = 118$)
NEAP, mEq/d	47.5 [4.6–61.1]	73.0 [61.2–86.5]	115.0 [86.5–300.0]	32.4 [12.0–43.3]	52.2 [43.5–62.9]	77.6 [63.4–149.6]
Age, y	53.9 ± 10.7	54.7 ± 10.7	54.0 ± 10.8	53.9 ± 11.2	51.8 ± 11.2	49.6 ± 12.0
Female	91 (39.6%)	82 (35.7%)	79 (34.5%)	51 (42.9%)	41 (34.5%)	43 (36.4%)
Black	230 (100.0%)	230 (100.0%)	229 (100.0%)	5 (4.2%)	9 (7.6%)	14 (11.9%)
mGFR, mL/(min · 1.73 m^2)	45.4 ± 12.9	45.7 ± 13.4	46.5 ± 13.2	36.1 ± 9.7	35.8 ± 10.8	36.4 ± 9.4
eGFR, mL/(min · 1.73 m^2)	42.2 ± 14.0	42.9 ± 13.8	42.6 ± 14.1	41.7 ± 12.7	40.0 ± 13.3	39.1 ± 11.3
UPCR, mg/g	368.9 ± 527.3	318.2 ± 549.6	313.5 ± 518.7	298.3 ± 967.7	446.5 ± 1053.4	416.9 ± 646.8
History of CVD	119 (51.7%)	113 (49.1%)	119 (52.0%)	4 (3.4%)	10 (8.4%)	6 (5.1%)
BMI, kg/m^2	30.2 ± 5.8	29.9 ± 6.3	30.4 ± 6.5	26.0 ± 3.6	27.0 ± 3.8	28.0 ± 4.6
Weight, kg	88.3 ± 18.0	88.3 ± 20.3	89.8 ± 20.6	75.2 ± 13.0	79.7 ± 14.9	83.9 ± 17.6
Smoking status						
Current smoker	61 (26.5%)	59 (25.7%)	73 (31.9%)	12 (10.1%)	7 (5.9%)	18 (15.3%)
Former smoker	68 (29.6%)	84 (36.5%)	66 (28.8%)	52 (43.7%)	50 (42.0%)	43 (36.4%)
Never smoker	101 (43.9%)	87 (37.8%)	90 (39.3%)	55 (46.2%)	62 (52.1%)	57 (48.3%)
BP medication[2]						
Ramipril	95 (41.3%)	92 (40.0%)	79 (34.5%)	N/A	N/A	N/A
Metoprolol	88 (38.3%)	91 (39.6%)	105 (45.9%)	N/A	N/A	N/A
Amlodipine	47 (20.4%)	47 (20.4%)	45 (19.7%)	N/A	N/A	N/A
BP goal[2]						
Low	112 (48.7%)	117 (50.9%)	108 (47.2%)	56 (47.1%)	58 (48.7%)	68 (57.6%)
Moderate	118 (51.3%)	113 (49.1%)	121 (52.8%)	63 (52.9%)	61 (51.3%)	50 (42.4%)
Protein intervention[2]						
Very low	N/A	N/A	N/A	4 (3.4%)	1 (0.8%)	1 (0.8%)
Low	N/A	N/A	N/A	85 (71.4%)	51 (42.9%)	33 (28.0%)
Usual	N/A	N/A	N/A	30 (25.2%)	67 (56.3%)	84 (71.2%)
Serum bicarbonate, mEq/L	25.5 ± 2.7	25.0 ± 2.7	24.8 ± 2.6	25.1 ± 3.4	24.6 ± 3.4	23.8 ± 3.3
Protein intake, g/d	69.5 ± 23.7	72.7 ± 21.9	75.5 ± 23.5	58.3 ± 17.8	74.5 ± 23.4	80.2 ± 20.3
Urine urea nitrogen, g/d	8.4 ± 3.4	8.9 ± 3.1	9.3 ± 3.4	7.0 ± 2.7	9.5 ± 3.6	10.3 ± 3.0
Urine potassium, mEq/d	68.7 ± 31.4	48.2 ± 15.7	34.0 ± 11.7	76.2 ± 23.4	65.3 ± 20.5	50.8 ± 14.3

[1] Values are mean ± SD for continuous variables and *n* (%) for categorical variables. For NEAP, values are mean [minimum-maximum]. AASK, African American Study of Kidney Disease and Hypertension; BP, blood pressure; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; MDRD, Modification of Diet in Renal Disease; mGFR, measured glomerular filtration rate; N/A, not applicable; NEAP, net endogenous acid production; UPCR, urine protein-to-creatinine ratio.
[2] BP medication, BP goal, and protein intervention refer to the randomly assigned groups in AASK (BP medication and BP goal) and the MDRD study (BP goal and protein intervention). N/A is used for randomly assigned groups that are not relevant to the specific study (protein intervention for AASK and BP medication for the MDRD study).

NEAP at the Bonferroni threshold ($0.05/757 = 6.6 \times 10^{-5}$) after adjusting for age, sex, race, randomization group (antihypertensive medication and blood pressure target), mGFR, log-transformed urine protein-to-creatinine ratio, history of cardiovascular disease, BMI, and smoking status (**Table 2**; **Supplemental Table 1**; **Figure 3**A). The categories of metabolites that were represented among the significant known serum metabolites included amino acids ($n = 6$), carbohydrates ($n = 3$), cofactors and vitamins ($n = 4$), 1 lipid, and xenobiotics ($n = 12$). When the model did not account for mGFR, there were 25 metabolites that were significantly associated with NEAP in AASK (Figure 3B).

In the MDRD study, 23 out of the 26 serum metabolites that were significantly associated with NEAP in AASK were detected. A total of 13 out of the 23 serum metabolites (57%) was significantly associated with NEAP in the MDRD study at the Bonferroni threshold ($0.05/23 = 2.2 \times 10^{-3}$) after adjusting for age, sex, race, randomization group (protein intake and blood pressure target), mGFR, log-transformed urine protein-to-creatinine ratio, history of cardiovascular disease, BMI, and smoking status. The 13 replicated metabolites included 5 amino acids (S-methylmethionine, indolepropionylglycine, indolepropionate, N-methylproline, N-δ-acetylornithine), 2 cofactors and vitamins [threonate, oxalate (ethanedioate)], 1 lipid (chiro-inositol), and 5 xenobiotics [tartronate (hydroxymalonate), catechol sulfate, hippurate, methyl glucopyranoside (α and β), and stachydrine] (Table 2; Figure 3A). The replicated metabolites represented a variety of subpathways: urea cycle; arginine and proline metabolism; tryptophan metabolism; methionine, cysteine, S-adenosylmethionine, and taurine metabolism; ascorbate and aldarate metabolism; inositol metabolism; food component/plant; benzoate metabolism; and bacterial/fungal. Higher levels of all 13 replicated metabolites were associated with lower NEAP (negative β-coefficients) in both AASK and the MDRD study. The correlation between the 13 replicated metabolites ranged from 0.08 to 0.82, with the highest correlation observed between stachydrine and N-methylproline (**Table 3**).

There was a significant difference in the ability of this panel of 13 replicated metabolites and covariates (age, sex, race, randomization group, mGFR, log-transformed urine protein-to-creatinine ratio, history of cardiovascular disease, BMI, and smoking status) to predict the highest of the NEAP quartiles, compared to a model with only the participant characteristics in both AASK (C-statistic for model without metabolites: 0.592; C-statistic for model with metabolites:

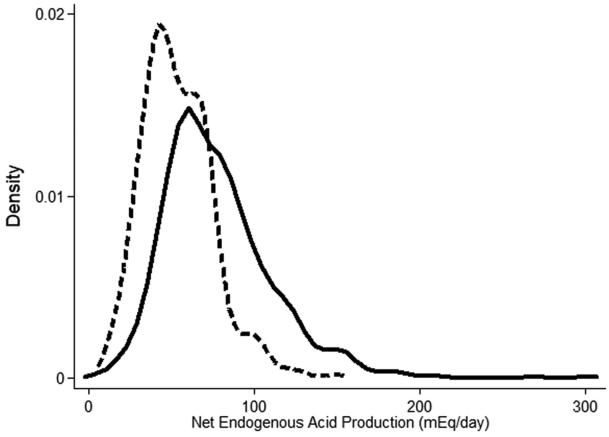

FIGURE 2 Kernel density plot of distribution of net endogenous acid production in AASK and the MDRD study. Solid line represents kernel density plot for AASK participants. Dashed line represents kernel density plot for MDRD participants. AASK, African American Study of Kidney Disease and Hypertension; MDRD, Modification of Diet in Renal Disease.

0.755; $P < 0.001$) and the MDRD study (C-statistic for model without metabolites: 0.733; C-statistic for model with metabolites: 0.806; $P < 0.001$).

Discussion

In the discovery study of 689 African-American adults with kidney disease and hypertension in AASK, we identified 26 out of 757 detected metabolites that were inversely associated with dietary acid load and independent of demographics, health behaviors and status, study design features, mGFR, and proteinuria. Thirteen of the identified metabolite associations replicated in an independent study population of 356 predominantly Caucasian adults with kidney disease in the MDRD study, including those involved in the metabolism of amino acids, cofactors and vitamins, lipids, and xenobiotics: methyl glucopyranoside (α and β), stachydrine, N-methylproline, catechol sulfate, indolepropionylglycine, N-δ-acetylornithine, S-methylmethionine, indolepropionate, hippurate, chiro-inositol, threonate, oxalate (ethanedioate), and tartronate (hydroxymalonate). This panel of 13 metabolites had a high cumulative ability to predict high dietary acid load beyond study design and participant characteristics in AASK and the MDRD study.

To the best of our knowledge, no prior research has studied metabolites associated with dietary acid load. However, many of the replicated metabolites found in the present study have been identified as biomarkers of other aspects of the diet. In a recent analysis of the DASH dietary pattern, we found that higher serum methyl-glucopyranoside (α and β), stachydrine, N-methylproline, and chiro-inositol were among the top 10 most influential metabolites for distinguishing between the DASH diet and control diet (26). N-δ-acetylornitine, S-methylmethionine, and catechol sulfate were also associated both with the DASH dietary pattern in the previous study and with dietary acid load in the present study. The concordance of the present study with our prior research on the DASH diet is consistent with previous reports that the DASH diet has a relatively low acid load (5). In terms of dietary components that these metabolites represent, methyl-glucopyranoside is a marker of total fruit intake, stachydrine and N-methylproline are markers of citrus fruit, and chiro-inositol is a component of phytic acid, which is present in many plant foods (27–30).

We also observed that higher serum levels of a couple of the metabolites associated with lower dietary acid load in the present study was associated with lower protein intake in a previous analysis of the MDRD study: indolepropionate and S-methylmethionine (31). Indolepropionate has also been reported to be inversely associated with consumption of red meat and eggs (30). We also found that indolepropionylglycine, which is similar to indolepropionate in that they are both involved in tryptophan metabolism, was associated with lower dietary acid load and has not been previously reported in the literature as a biomarker of dietary intake. S-methylmethionine,

TABLE 2 Serum known metabolites significantly associated with net endogenous acid production in AASK and the MDRD study[1]

			AASK			MDRD		
Superpathway	Subpathway	Metabolite	β[2]	SE	*P* value	β[2]	SE	*P* value
Amino acid	Methionine, cysteine, SAM, and taurine metabolism	S-methylmethionine	−0.054	0.012	5.06×10^{-06}	−0.186	0.047	8.18×10^{-05}
Amino acid	Tryptophan metabolism	Indolepropionylglycine	−0.061	0.012	2.25×10^{-07}	−0.144	0.032	6.90×10^{-06}
Amino acid	Tryptophan metabolism	Indolepropionate	−0.061	0.011	6.65×10^{-08}	−0.109	0.027	8.34×10^{-05}
Amino acid	Urea cycle; arginine and proline metabolism	N-methylproline	−0.120	0.015	3.34×10^{-15}	−0.172	0.032	9.15×10^{-08}
Amino acid	Urea cycle; arginine and proline metabolism	N-δ-acetylornithine	−0.043	0.006	8.29×10^{-12}	−0.071	0.016	1.31×10^{-05}
Cofactors and vitamins	Ascorbate and aldarate metabolism	Threonate	−0.048	0.006	5.13×10^{-15}	−0.044	0.013	1.12×10^{-03}
Cofactors and vitamins	Ascorbate and aldarate metabolism	Oxalate (ethanedioate)	−0.041	0.005	9.72×10^{-16}	−0.033	0.010	1.14×10^{-03}
Lipid	Inositol metabolism	Chiro-inositol	−0.145	0.020	4.13×10^{-13}	−0.127	0.035	3.38×10^{-04}
Xenobiotics	Bacterial/fungal	Tartronate (hydroxymalonate)	−0.079	0.009	2.45×10^{-17}	−0.038	0.012	1.19×10^{-03}
Xenobiotics	Benzoate metabolism	Catechol sulfate	−0.039	0.008	3.84×10^{-06}	−0.104	0.020	2.36×10^{-07}
Xenobiotics	Benzoate metabolism	Hippurate	−0.042	0.010	1.33×10^{-05}	−0.086	0.024	3.15×10^{-04}
Xenobiotics	Food component/plant	Methyl glucopyranoside ($\alpha + \beta$)	−0.118	0.012	8.04×10^{-22}	−0.156	0.025	1.82×10^{-09}
Xenobiotics	Food component/plant	Stachydrine	−0.123	0.015	6.40×10^{-16}	−0.188	0.034	8.86×10^{-08}

[1]Statistical significance was assessed using Bonferroni method to account for multiple comparisons (AASK: $P < 6.6 \times 10^{-5}$; MDRD: $P < 2.2 \times 10^{-3}$). AASK, African American Study of Kidney Disease and Hypertension; MDRD, Modification of Diet in Renal Disease.
[2]β-coefficients are expressed per 10 mEq/d higher net endogenous acid production.

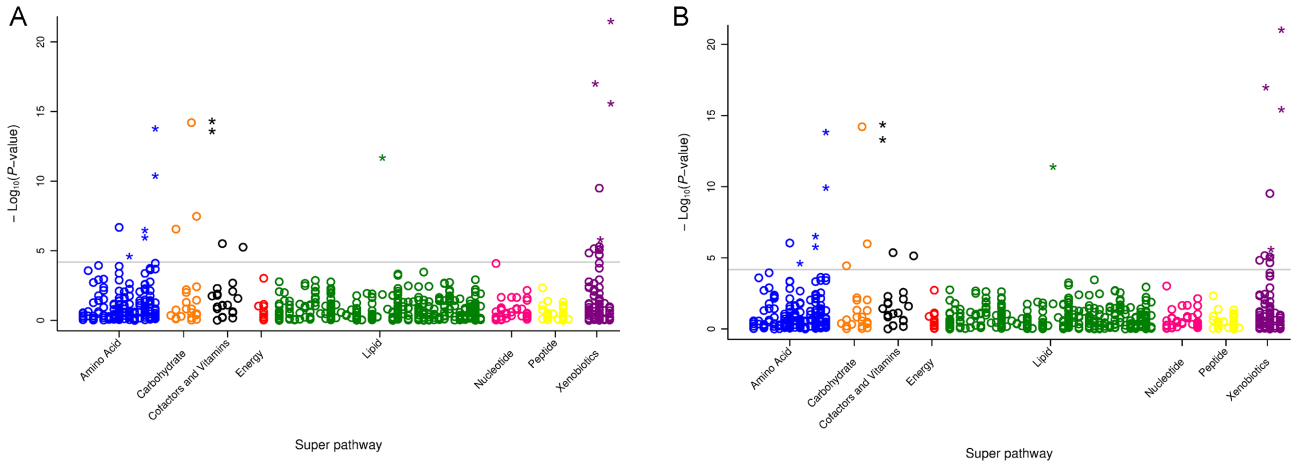

FIGURE 3 Plot of $-\text{Log}_{10}(P$ values) for the adjusted association between serum known metabolites and net endogenous acid production in AASK according to metabolic pathway with adjustment for mGFR (A) and without adjustment for mGFR (B). *P* values were calculated from multivariable linear regression models adjusted for age, sex, race, randomly assigned intervention group (antihypertensive medication and blood pressure control target), log-transformed urine protein-to-creatinine ratio, history of cardiovascular disease, BMI, and smoking status. For (A), models were also adjusted for mGFR. Asterisks denote metabolites that replicated in the MDRD study. The solid black line represents the Bonferroni threshold for determining statistical significance in AASK ($0.05/757 = 6.6 \times 10^{-5}$). AASK, African American Study of Kidney Disease and Hypertension; MDRD, Modification of Diet in Renal Disease; mGFR, measured glomerular filtration rate.

in addition to being inversely associated with dietary protein, is detected in cruciferous vegetables, such as cabbage and corn (31–33).

Four additional metabolites were identified in our study as novel markers of dietary acid load: hippurate, threonate, oxalate (ethanedioate), and tartronate (hydroxymalonate). Higher blood levels of hippurate are associated with consumption of fruit and whole grains, and it is formed in the gut through metabolism of phenolic compounds (34, 35). Both oxalate and threonate are degradation products from metabolism of ascorbic acid, and therefore serve as markers of plant foods containing vitamin C (27, 30, 36). Higher blood levels of threonate has been related to dietary intake of green leafy vegetables (29). To the best of our knowledge, tartronate has not been reported as a diet biomarker. It may be similar to threonate and oxalate because tartronate semialdehyde is also involved in ascorbate and aldarate metabolism.

Overall, we found 13 metabolites that were significantly associated with dietary acid load in 2 independent study populations, including both established and more novel diet biomarkers. An innovative approach of the present study was to relate the serum metabolome to dietary acid load, which is a biologically relevant and holistic measure of acid-producing foods, such as meats and cheese, and base-producing foods,

TABLE 3 Pearson correlation coefficients for 13 serum known metabolites significantly associated with net endogenous acid production in AASK and the MDRD study[1]

	N-δ-acetylornithine	N-methylproline	S-methylmethionine	Indolepropionate	Indolepropionylglycine	Oxalate (ethanedioate)	Threonate	Chiro-inositol	Catechol sulfate	Hippurate	Methyl glucopyranoside ($\alpha + \beta$)	Stachydrine	Tartronate (hydroxymalonate)
N-δ-acetylornithine	1.00	—	—	—	—	—	—	—	—	—	—	—	—
N-methylproline	0.37	1.00	—	—	—	—	—	—	—	—	—	—	—
S-methylmethionine	0.12	0.14	1.00	—	—	—	—	—	—	—	—	—	—
Indolepropionate	0.21	0.09	0.21	1.00	—	—	—	—	—	—	—	—	—
Indolepropionylglycine	0.33	0.10	0.17	0.58	1.00	—	—	—	—	—	—	—	—
Oxalate (ethanedioate)	0.24	0.39	0.14	0.11	0.12	1.00	—	—	—	—	—	—	—
Threonate	0.36	0.38	0.14	0.15	0.21	0.76	1.00	—	—	—	—	—	—
Chiro-inositol	0.42	0.78	0.14	0.10	0.22	0.36	0.40	1.00	—	—	—	—	—
Catechol sulfate	0.30	0.10	0.19	0.32	0.34	0.13	0.25	0.19	1.00	—	—	—	—
Hippurate	0.27	0.14	0.12	0.29	0.26	0.14	0.25	0.24	0.57	1.00	—	—	—
Methyl glucopyranoside ($\alpha + \beta$)	0.45	0.73	0.21	0.17	0.23	0.47	0.46	0.67	0.23	0.21	1.00	—	—
Stachydrine	0.44	0.82	0.13	0.08	0.15	0.45	0.45	0.71	0.17	0.17	0.68	1.00	—
Tartronate (hydroxymalonate)	0.22	0.38	0.14	0.15	0.15	0.75	0.64	0.34	0.13	0.12	0.46	0.42	1.00

[1] $P < 0.001$ for all correlation coefficients. AASK, African American Study of Kidney Disease and Hypertension; MDRD, Modification of Diet in Renal Disease.

such as fruits and vegetables, in the diet. It was expected and promising that these 13 significant, replicated metabolites have previously been shown to represent aspects of the diet and metabolic pathways related to acid load, for example, the DASH dietary pattern, protein, fruits, and vegetables. We found that these 13 metabolites together significantly improved the ability to classify high compared with low dietary acid load. As such, these findings suggest the utility of a multimarker panel of metabolites to reflect the multidimensional nature of dietary acid load.

In the present study, adjustment for mGFR did not substantially change the results in terms of the number of significant metabolites, strength and precision of the estimates, and significance levels. Prior research has demonstrated the extent to which the metabolome reflects accumulation of compounds in the blood in those with impaired kidney function, i.e. decreased GFR (37, 38). It is particularly critical to adjust for mGFR in analyses of metabolites and chronic kidney disease progression, but it is perhaps less critical to do so in cross-sectional analyses with exposures relatively unrelated to kidney disease status, for example, dietary intake. Nonetheless, given that there was some variability in baseline mGFR, all of our main analyses were adjusted for mGFR.

There are several noteworthy strengths of our study. We used a broad platform consisting of a wide range of compounds to profile the metabolome, which maximizes the opportunity for discovery of novel biomarkers of dietary acid load. In addition, we estimated dietary acid load using objective measures, that is, urine concentration of urea nitrogen to estimate protein and urine potassium, rather than more error-prone self-reported dietary intake. We were able to leverage a secondary dataset generated by the same metabolomic platform and which was conducted in another, independent study population of kidney disease patients (i.e., the MDRD study) to replicate the study findings from the discovery subset of AASK participants.

There are also some limitations to acknowledge. The replication sample differed from the discovery sample with respect to baseline characteristics (race, health behaviors, health history), which could in part explain why not all of the metabolites replicated. In addition, the range of dietary acid load values also varied between the 2 studies, potentially because of the protein reduction intervention given that NEAP and metabolites were measured at the 12-mo visit in the MDRD study, whereas data were obtained at baseline in AASK. Residual confounding from lack of measurement or imprecise measurement of confounders could partially explain the findings. However, we were able to adjust for several relevant demographic characteristics, health behaviors and status, mGFR, proteinuria, and randomization group. Each metabolite was listed as belonging to a single metabolic superpathway and subpathway, whereas they could be appropriately classified in a different manner. For example, although S-methylmethionine was reported as an amino acid and specifically related to methionine, cysteine, S-adenosyl methionine and taurine metabolism, perhaps more relevant is that it is a potential biomarker of cruciferous vegetable consumption (31–33). Nonetheless, we used the pathway designation for the sake of consistency of reporting results from this platform.

In summary, a metabolomic profile of serum specimens from kidney disease patients in 2 study populations identified 13 replicated metabolites associated with dietary acid load, after accounting for demographics, study design, health behavior, health status, mGFR, and proteinuria. Additional research is warranted to validate that these compounds represent dietary acid load in healthy populations, and to assess the relation between these compounds and clinical outcomes. This panel of 13 serum compounds may be used as a potential objective marker of dietary acid load in future nutrition research studies.

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